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Effect of buprofezin on the growth, development and morphometric parameters of *Spodoptera litura* (Fabricius) (Order: Lepidoptera, Family: Noctuidae) adult

Mst. Rokeya Khatun[1]✉, Gopal Das[1], Kazi Shahanara Ahmed[1], Hisashi Kato-Noguchi[2]

[1]Department of Entomology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[2]Department of Applied Biological Science, Kagawa University, Japan

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Correspondence:
Mst. Rokeya Khatun
✉: rokeya.entom@bau.edu.bd

ABSTRACT

The experiments were conducted to know the efficacy of Buprofezin (Award 40 SC), an insect growth regulator against 2nd instar larvae of *Spodoptera litura* (Fabricius) under laboratory conditions (insect rearing was done at room temperature and relative humidity that is 26⁰C-30⁰C and 65-80% relative humidity) at three different concentrations viz. 200, 400, and 600 ppm through three different application methods like direct or topical, indirect or leaf-dip and combined. The experiment was laid out in complete randomized design (CRD) with three replications and ten 2nd instar larvae of *Spodoptera litura* were used for each replication. Results revealed that this growth regulator strongly arrested the growth and development of *S. litura* (Fabricius) from larval stage to adult. The treated data were recorded after adult emergence and compared with untreated control adult. The results clearly showed that Buprofezin had significant effect on the inhibition of adult weight; wing length and width compared to control and consequence were clearly dose, time and method dependent. As well as we are hopeful because we found some abnormal adult from the treated larvae that may increase the potentiality of Buprofezin as an excellent tool of IPM. The maximum, 54.53% adult weight reduction was recorded from 600 ppm of Buprofezin through combined application method which was followed by leaf-dip (46.41%) and topical application method (39.86%). Similarly, 23.13% wing length and 22.27% wing width were inhibited when larvae were treated with 600 ppm through combined application method compared to control. The 2nd best result was found from 400 ppm and leaf-dip application method. The dose, 200 ppm and topical application method was found to be less effective compared to the rest of the two doses or two application methods. So this is the demand of the current situation to evaluate this selective bio-rational molecules against *S. litura* as the alternatives of conventional insecticides to combat resistance development, effective larval control, inhibition of growth and development, to keep the natural enemies safe and fit these molecules in IPM packages individually or combinedly.

Introduction

Among the polyphagous insects pest *Spodoptera litura* (Fabricius), (Lepidoptera: Noctuidae) have attained the status of one of the key insect pest because of its infestation level (Brown and Dewhurst, 1975; Holloway, 1989). It attacks various crops of more than 120 host plants including cereals, vegetables, weeds and ornamental plants (Ramana *et al*., 1988). *Spodoptera litura* is the most deleterious insect pest that causes heavy losses in many agricultural crops including tobacco, tomato, cotton, chilly, okra, cauliflower, castor, groundnut, soybean, maize and black gram etc. thus, deprives the farmers from getting higher yield (CABI, 2009). The leaf worm, *S. litura* (Fabricius), a serious but sporadic insect pest and this insect have the ability of causing economic losses from 25.8-100% based on crop stage and its infestation level in the field (Dhir *et al*., 1992). Some controlled experiments on soybeans in India showed that, crops chemically protected from *S. litura* and other pests yielded over 42% more than crops which were not sprayed (Srivastava *et al*., 1972). On tobacco, in India, it was estimated that two, four and eight larvae per plant reduced yield by 23-24, 44.2 and 50.4%, respectively (Patel *et al*., 1971). On *Colocasia esculenta*, an average of 4.8, 4th-instar larvae per plant reduced yield by 10%, while 2.3 and 1.5 larvae reduced yield of aubergines (eggplant) and capsicum in glasshouses by 10% also (Nakasuji *et al*., 1977).

To get rid from this notorious insect farmers are solely dependent on synthetic insecticides but it has already been reported that *S litura* (Fabricius) has developed high level of resistance to almost all conventional

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insecticides due to intensive and injudicious use of broad-spectrum insecticides (Kranthi *et al*., 2002). The development of resistance to synthetic chemicals like cypermethrin, fenvalerate and quinalphos (Ramakrishnan *et al*., 1984; Naeem Abbas *et al*., 2014) prompted the scientific community to look for alternative methods for the management of this insect. At this critical time period uses of target based/ selective bio-degradable insecticides such as compounds based on bacteria, fungi, insect growth regulators and botanical pesticides (Nakasuji *et al*., 1977; Sharma *et al*., 2008; Wang, 2009) may prove worthy for control of *Spodoptera litura* (Fabricius) than broad-spectrum insecticides. Insect growth regulators (IGRs) are potent one chemical to combat this problem. There are different types of insect growth regulators among them, In general, IGRs, which act as chitin synthesis inhibitors have been regarded as excellent integrated control insecticides because of their specificity to the target pest, their relative non-toxicity to beneficial organisms and their general safety to vertebrates, mollusks and plants (Deakle, 1982; Frank, 1978; Horn, 1988; Wilkinson *et al*., 1978). Among different IGRs Buprofezin is one of the important chitin synthesis inhibitors (CSIs) that prevent chitin bio-synthesis and deposition in insect body. As a result, new cuticle formation is arrested and old cuticles become fractured and insect finally die (Gelbic *et al*., 2011; Mulder, 1973).

If the insects are not died its growth and development becomes hampered. Buprofezin reduces the population of *S. litura* by inhibiting the bio-synthesis of chitin during and after moulting process and ultimately disrupt the growth and development of this insect from its neonate stage to adult and also have feeding inhibition that reduces the growth of the insect (Manal, 2012). Buprofezin also affects the population of *S. litura* by reducing fecundity, egg hatchability, egg sterility, production of abnormal larvae and pupae (Ragaei, 2011). Buprofezin was found to be effective against hemipteran pests, some lepidopteran larvae, spiders etc. (Deng *et al*., 2008; Izawa *et al*., 1985; Nagata, 1986).

IGRs compounds are noble crop protectants insecticides and growth inhibitors, which inhibits or prevents normal metamorphosis of immature stages to the adult stage. These compounds have been tested successfully against several insect species e.g. *S. litura* (Wang, 2009) and *S. littoralis* (Gelbic *et al*., 2011). In Bangladesh Buprofezin, potent chitin synthesis inhibitor (CSI) brings to table as possible alternatives of broad-spectrum insecticides in integration with other components of integrated pest management (IPM) for proper control of *S. litura* (Fabricius). This experiment was done to find out the efficacy of Buprofezin on certain morphological aspects of *S. litura* larvae for managing this insect as suitable means for use in programs of integrated pest management (IPM).

Materials and Methods

The experiments were conducted in the Entomology laboratory of Bangladesh Agricultural University, Mymensingh, Bangladesh from July 2015 to June 2016.

Mass rearing of Spodoptera litura

The egg masses of *Spodoptera litura* (Fabricius) were collected from soybean field and were kept in petridishes (150 mm x 100 mm, height 25 mm, Anumbra, Germany) for hatching. After hatching, fresh soybean leaves were supplied to the neonate larvae for feeding and this helped the larvae for its proper growth. This feeding was continued until the larvae became matured and when the larvae reached to the final instar, they were transferred to the plastic container (medium sized plastic box, RFL company, Bangladesh) filled with soil for pupation. After emergence of adult from pupa, male & and female (A large yellowish or light brown patch on the median area adjacent to inner margin. This patch is not present in females) moth was kept in a rearing chamber at room temperature and relative humidity that is 26 ^{0}C-30 ^{0}C and 65-80% relative humidity with previously growth aroid plants. After mating, female moths laid eggs in masses on the lower and upper surface of the aroid leaves. The leaves containing egg masses were cut and then kept in sterilized petridishes with wet cotton to prevent the drying of leaves. After 3-4 days, the eggs were hatched and neonate larvae were come out. Fresh and insecticides free soybean leaves were provided every day for larval rearing. When the larvae reached to 2nd instar with uniform size were used for treatment applications. Rearing was continued until the end of the experiments to get sufficient larvae for the experiments.

Specifications of treatments

In the laboratory, three treatments of Buprofezin, Award 40 SC (0.25 L/ha for brown planthopper, Square Pharmaceuticals limited, Bangladesh) viz., 200, 400 and 600 ppm along with control were used in different application methods. Each treatment was replicated thrice and ten 2nd instar larvae (1-2 days old larvae) of *S. litura* (Fabricius) were used for each replication.

Methods of treatment application

For this experiment, three different application methods were applied. Descriptions are presented below:

Topical application method (Direct method)

In this method, the larvae were directly treated (using micropipette; Eppendorf micropipette, 100-1000 micro litre, Research plus, Germany) with different concentrations of Award 40 SC (Buprofezin).

Then the treated larvae were immediately transferred into a sterilized petridish (150mmx100mm, height 25 mm, Anumbra, Germany) using a sterilized fine brush. In this case, fresh soybean leaves were supplied into the petridish for feeding the larvae. A moist filter paper was placed into the petridish to avoid desiccation. Then the petridish was covered with sterilized lid allowing proper air circulation and avoiding larvae from escape. As the larvae became bigger in size later it was in a sterilized plastic box (medium sized plastic box, RFL company, Bangladesh) with lid that was perforated (cut manually with a size of 100x100 mm square shape) and covered with net (0.1 mm mesh size fabric net) avoiding larvae from escape.

Leaf-dip method (Indirect method)

In this case, soybean leaves (4-5 leaves that was dipped about 5 minutes in different solutions) (Mass rearing was done in aroid plants but we used the soybean leaves as an experimental arena; because aroid leaves are more waxy and it can't hold water) were treated with Award 40 SC (Buprofezin) with different concentrations as experimental specifications (200, 400 and 600 ppm). Following that the treated leaves were kept open in the air to avoid wetting of the leaves. The proper dried leaves were placed on moist filter paper (to prevent the desiccation of the leaves) in a sterilized petridish. Then the untreated larvae of definite number were transferred into the sterilized (70% ethanol) petridish using fine sterilized brush and covered with sterilized lid. Later, the same procedures were followed as the larvae became bigger in size that was done in case of topical application method.

Combined method (Direct method + indirect method)

In case of combined method, both larvae and soybean leaves were treated with different concentrations of Award 40 SC (Buprofezin). After that the treated leaves were properly dried and placed on moist filter paper in a sterilized petridish. The petridishes and brushes, hand and working surface that were used in this experiment all were sterilized with alcoholic solution (70% ethanol) before work. The treated larvae were then transferred on treated leaves using fine brush and covered with sterilized lid. Next procedures were same as followed in direct and indirect method.

Data collection

Data of adult weight and wing length and width (after emergence of adult from pupa) were recorded with the help of the scale (15 cm Faber-Castell scales, Germany) after the emergence of adult from the pupa. As the 2nd larval instar was treated according to the experimental design, died larvae were separated and alive larvae were further provided with either fresh or treated soybean leaves based on treatment application method and rearing was continued until the adult emergence. The percentage of adult weight, and wing length and width reduction were calculated using the following formulae;

% Adult weight reduction = (Po – Pr)/ Po x 100

Where,
Po = Mean weight of a single adult in control condition
Pr = Mean weight of a single adult in a treated condition

% Adult wing length/ width inhibition = (Po – Pr)/ Po x 100

Where,
Po = Mean length/width of a single adult wing in control treatment
Pr = Mean length/width of a single adult wing in a specific treatment

Statistical analysis

The recorded data were compiled and tabulated for statistical analysis. Analysis of variance (ANOVA) was done with the help of MSTAT-C program. The mean differences among the treatments were adjudged with Duncan`s Multiple Range Test (DMRT) and Least Significant Difference (LSD).

Results and Discussion

Efficacy of Buprofezin on weight reduction of Spodoptera litura adult

Topical application method

(Buprofezin is one of the important IGR that has significant effect on the growth and development of insects and in this experiment adult weight reduction due to topical application of different concentrations of Buprofezin is shown in (Table 1). Buprofezin was significantly ($P<0.01$) reduced the weight of adult *S. litura*. As the larvae treated with Buprofezin it's being accumulated in the insect body and ultimately hampered the adult growth. The lowest mean adult weight 39.70 mg/adult was recorded from 600 ppm which was followed by 400 ppm (52.30 mg/adult) and 200 ppm (62.06 mg/adult). Maximum mean adult weight reduction 39.86% recorded from 600 ppm followed by 400 ppm (20.78%) and 200 ppm (6.38%) that was statistically significant ($P<0.01$). From the experimental results it was cleared that adult weight reduction was dose dependent as the dose increased adult weight was decreased.

These findings could be linked with Wang and Tian (2009) and Gelbic *et al*. (2011) who successfully tested IGRs compounds against several insect species example *Spodoptera litura* and *S. littoralis* which inhibits or prevents normal metamorphosis of immature stages to the adult stages.

Leaf-dip or indirect application method

Buprofezin had significant (P<0.01) and dose dependent effects on the weight reduction of adult *S. litura* (Table 1). Indirect application method was more effective for adult mean weight reduction than the direct application method. The lowest mean adult weight was 37.56 mg/adult obtained from 600 ppm followed by 51.08 mg/adult from 400 ppm and 56.52 mg/adult from 200 ppm. In this method maximum mean adult weight reduction 46.41% was recorded from 600 ppm (P<0.01) which was followed by 400 ppm (P<0.01) 27.13% and 200 ppm (P<0.01) 19.37% compared to control. The results clearly revealed that Buprofezin had significant effect on adult weight reduction. In direct (topical) application method maximum mean adult weight reduction was 39.86% but in indirect (leaf-dip) method, it was 46.41% that means indirect effect was more effective than direct one for mean adult weight reduction of *S. litura*.

Table 1. Effects of different treatments of Buprofezin on the weight changes of *S. litura* adult through topical, leaf-dip, and combined application methods

Application methods	Treatments		Weight (mg/adult)	% reduction over control
Direct	Award 40 SC @ 200 ppm		62.06b	6.38
	Award 40 SC @ 400 ppm		52.30c	20.7
	Award 40 SC @ 600 ppm		39.70d	39.86
	Control		66.02a	---
	SD	11.73		
	P-level	**		
	CV (%)	0.89		
Indirect	Award 40 SC @ 200 ppm		56.52b	19.37
	Award 40 SC @ 400 ppm		51.08c	27.13
	Award 40 SC @ 600 ppm		37.56d	46.41
	Control		70.10a	---
	SD	13.47		
	P-level	**		
	CV (%)	0.04		
Combined	Award 40 SC @ 200 ppm		54.29b	22.27
	Award 40 SC @ 400 ppm		50.10c	28.27
	Award 40 SC @ 600 ppm		31.76d	54.53
	Control		69.85a	---
	SD	15.66		
	P-level	**		
	CV (%)	0.07		

In a column, means of similar letter (s) do not differ significantly. DAT = Days After Treatment, ** = Significant at 1% level of probability, P-level = Probability Level, CV = Co-efficient of Variation, SD = Standard Deviation.

Combined application method

The highest adult weight reduction was recorded when both the larvae and soybean leaves were treated with Buprofezin (Table 1). The lowest mean adult weight 31.76 mg/adult was observed from 600 ppm followed by 50.10 mg/adult from 400 ppm and 54.29 mg/adult from 200 ppm. From combined method of application, maximum mean adult weight reduction 54.53% was observed from 600 ppm (P<0.01) followed by 28.27% (400 ppm) and 22.27% (200 ppm) compared to control that was statistically significant (P<0.01). In direct application method maximum mean adult weight reduction (39.86%) and indirect (46.41%) was obtained from 600 ppm but due to combined application it was 54.53%. According to the experimental results, direct application method and low dose 200 ppm of Buprofezin was less effective for adult weight reduction but the effectiveness was gradually increased with the increasing of dose 400 ppm and 600 ppm and time. From this result it was also cleared that indirect application method was more effective than direct application method but the combination application method was the best as it was more effective for adult weight reduction compared to individual one.

Efficacy of Buprofezin on wing length and width inhibition of S. litura adult

Topical application method

Buprofezin had dose dependent effect on the wing length and width inhibition of adult *Spodoptera litura* (Table 2). The length and width of wing gradually decreased with increasing concentrations level of Buprofezin and time. Among different concentrations the maximum mean wing length and width inhibitions were observed from 600 ppm 14.80% and 14.39% which was followed by 400 ppm 12.53% and 10.79% and 200 ppm 10.56% and 6.89% that was statistically significant (P<0.01). So, Buprofezin had significant effect on the wing length and width inhibition as the larvae directly treated with Buprofezin.

Leaf-dip application method

Adult wing length and width gradually decreased with the increased concentrations of Buprofezin. In the leaf-dip method, larvae were fed the Buprofezin treated soybean leaves with different concentrations that accumulated in insect body and ultimately reduced the wing length and width of adult *S. litura*. Among different concentrations, the maximum mean wing length and width inhibition was observed from 600 ppm 16.74% and 16.47% which was followed by 400 ppm 14.12% and 13.67% and 200 ppm 9.65% and 11.32% over control that was statistically significant (P<0.01, Table 3). In topical application method maximum mean wing length and width inhibition was 14.80% and 14.39% recorded from 600 ppm but in leaf dip method it was 16.74% and 16.47% greater than topical application method. That's why leaf-dip application method was more effective than topical application method.

Table 2. Effects of different treatments of Buprofezin on adult wing length and width of *Spodoptera litura* adult through topical application method

Treatments	Length of wing(mm)	% inhibition over control	Width of wing (mm)	% inhibition over control
Award 40SC@ 200 ppm	14.98b	10.56	6.21b	6.89
Award 40 SC@400 ppm	14.65c	12.53	5.95c	10.79
Award 40SC@600 ppm	14.27d	14.80	5.71d	14.39
Control	16.75a	---	6.67a	---
SD	1.10		0.41	
P-level	**		**	
CV (%)	0.20		0.29	

In a column, means of similar letter (s) do not differ significantly. DAT = Days After Treatment, ** = Significant at 1% level of probability, P-level = Probability Level, CV = Co-efficient of Variation, SD = Standard Deviation.

Table 3. Effect of different treatments of Buprofezin on adult wing length and width of *Spodoptera litura* adult through indirect application method

Treatments	Length of wing (mm)	% inhibition over control	Width of wing(mm)	% inhibition over control
Award 40SC@200 ppm	15.16b	9.65	6.03b	11.32
Award 40 SC@400 ppm	14.41c	14.12	5.87c	13.67
Award 40 SC@600 ppm	13.97d	16.74	5.68d	16.47
Control	16.78a	---	6.80a	---
SD	1.24		0.49	
P-level	**		**	
CV (%)	0.16		0.18	

In a column, means of similar letter (s) do not differ significantly. DAT = Days After Treatment, ** = Significant at 1% level of probability, P-level = Probability Level, CV = Co-efficient of Variation, SD = Standard Deviation

Combined application method

The highest adult mean wing length and width inhibition was observed when both the larvae and soybean leaves were treated with different concentrations of Buprofezin is shown in Table 4. The lowest wing length and width were recorded from 600 ppm that was 12.89 mm and 5.27 mm which was followed by 13.38 mm and 5.43 mm from 400 ppm and 13.92 mm and 5.66 mm from 200 ppm. The maximum 23.13% and 22.27% mean adult wing length and width inhibition was recorded from 600 ppm which was followed by 400 ppm 20.21% & 19.91% and 200 ppm 16.99% and 16.51% that was statistically significant (P<0.01) compared to control. Maximum mean adult wing length and width inhibition from topical application method was observed from 600 ppm (P<0.01) 14.80% and 14.39%, in leaf-dip method it was 16.74% and 16.47% and in combined method it was 23.13% and 22.27% which was greater than two individual methods. From the experimental results it was cleared although all types of abnormalities like weight reduction, wing length and width inhibition of adult were dose dependent and combined application method was more effective for these abnormalities than the direct & indirect application method.

Efficacy of Buprofezin on abnormalities of Spodoptera litura adult

Topical application method

Different concentrations of Award 40 SC Buprofezin applied directly on 2nd instar larvae of *S. litura* to observe the inhibited growth and development of larva, pupa and adult. In case of adult, weight, wing length and width and abnormalities (Plate 1) were observed. From this application method (Plate 1) maximum 14.12% abnormal adult was recorded from 600 ppm followed by 12.67% (400 ppm) and 5.78% (200 ppm) respectively compared to control (2.00%). Adult abnormalities were dose-dependent; this was cleared from this result.

Table 4. Effect of different treatments of Buprofezin on adult wing length and width of *Spodoptera litura* adult through combined application method

Treatments	Length of wing (mm)	% inhibition over control	Width of wing(mm)	% inhibition over control
Award 40SC@200 ppm	13.92b	16.99	5.66b	16.51
Award 40SC@400 ppm	13.38c	20.21	5.43c	19.91
Award 40SC@600 ppm	12.89d	23.13	5.27d	22.27
Control	16.77a	---	6.78a	----
SD	1.74		0.68	
P-level	**		**	
CV (%)	0.17		0.33	

In a column, means of similar letter (s) do not differ significantly. DAT = Days After Treatment, ** = Significant at 1% level of probability, P-level = Probability Level, CV = Co-efficient of Variation, SD = Standard Deviation

[A] [B] [C]

Plate 1. Representative photographs of *S. litura* adults. Deformed adults (A-B) and normal adult (C). Deformed adults were developed when larvae were treated with 600 ppm of Buprofezin

Leaf-dip application method

When the soybean leaves treated with different concentrations of Award 40 SC and then it supplied to the untreated 2nd instar larvae of *S. litura* then it was observed that percent abnormal adult increased than the topical application method shown (Plate 1). Maximum abnormal adult 20.34% was recorded from 600 ppm followed by 15.51% (400 ppm) and 7.78% (200 ppm) respectively in comparison with control (2.00%). From this experiment, it was cleared that maximum abnormal adult 20.34% (600 ppm) was found from indirect application method that was higher than direct (14.12%) application method and it was also cleared that in leaf-dip application method was more effective than topical application method for adult abnormalities of *Spodoptera litura.*

Combined application method

The highest abnormal adult 24.89% was found from 600 ppm followed by 400 and 200 ppm 18.12% and 10.12%, respectively when both the larvae and soybean leaves were treated with different concentrations of Buprofezin (Plate 1) and it was higher than the topical and leaf-dip application methods. So, from these results it was cleared that the highest percent of abnormal adults were found from combined application method than the individual one compared to control. Due to different concentrations of Buprofezin application adult wouldn't come out completely from the pupa and it remained in an intermediate condition (not a pupa or not an adult) and if any adult came out it become deformed. Sometimes adult wing became crinkled and adult became small sized.

These results agreed with the results of Wang and Tian (2009) and Gelbic *et al*. (2011) who successfully tested IGRs compounds on *Spodoptera litura* and *S. littoralis* and found that this IGRs product inhibits or prevents normal metamorphosis of immature stages to the adult stages of these two insect species.

Conclusion

This study clearly indicated that Buprofezin significantly inhibited the growth of adult *S. litura* regarding adult weight reduction, wing length and width inhibition and abnormalities of adult. The highest percentage of adult weight reduction and over control was found from 600 ppm of Buprofezin through different application methods followed by 400 ppm and 200 ppm in case of topical, leaf-dip and combination application methods This study also revealed that abnormalities of pupa and adult were also dose-dependent as the concentration of Buprofezin increased abnormalities was also increased. Maximum percentage of abnormal pupa and adult was recorded from 600 ppm and combined application method followed by 400 ppm and leaf-dip method and then lowest 200 ppm and topical application method compared to control.

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